SOME GENERAL REGULARITIES IN THE FORMATION OF L-FORMS IN VARIOUS PATHOGENIC SPECIES OF BACTERIA

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UNITED STATES ARMY
BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

Translated by Sp/6 Charles T. Ostertag, Jr.

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SOME GENERAL REGULARITIES IN THE FORMATION OF L-FORMS IN VARIOUS PATHOGENIC SPECIES OF BACTERIA

[Following is the translation of an article by G. Ya. Kagan, S. V. Prozorovskiy, Ye. I. Koptelova, A. G. Shchegolev, V. T. Savenkova, V. S. Levashev, Z. A. Pesina, and V. S. Mikhaylova, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR published in the Russian-language periodical, Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No 11, 1963, page7-12. It was submitted on 5 Jul 1962. Translation performed by Sp/6 Charles T. Ostertag Jr.]

The establishment of the existence of L-forms considerably widened our concepts concerning the extent of bacterial mutation. At the present time the morphological and physiological peculiarities of these forms have been well studied. However there are few facts which permit the forming of a specific opinion concerning the mechanisms for the examination of L-forms and their reversion into bacterial cultures.

Mention must be made of the complete agreement of opinions on the mechanism of formation of unstable L-forms which are considered as modifications of bacterial forms which have lost the usual morphological features of cells and colonies and which are formed and maintained under the influence of specific agents and under specific conditions of cultivation. It is considerably more difficult to visualize the formation of stable L-forms which as it will be shown below may develop from unstable modified forms or may be formed directly from bacterial forms. Stable L-forms are hereditarily fixed forms of mutability, however up until now the problem remains unexplained whether their origination can be considered a process caused by gene mutations or if it is the factor causing the hereditary changes characteristic for L-forms connected only with changing conditions of the medium and consequently with a change of metabolism.

With the aim of explaining the probable mechanisms of formation of L-forms and their reversion into bacteria we considered it necessary to analyze the experimental data characterizing the formation of L-forms in various species, strains and populations, and to examine the forms of reactions of the bacterial populations of various species of bacteria in response to L-transforming actions.

Transformation of bacteria into L-forms depends on many conditions. The first of these is the species of bacteria. Thus according to data by Terranova and de Gregorio the most expressed capability for L-transformation is possessed by Streptobacillus moniliformis, Bacteroides and Proteus vulgaris, and to a lesser degree by representatives of the salmonella group. When determining the ratio of the overall number of bacterial cells to the number of cells which form L-forms, the so-called B/L coefficient explains that in proteus it fluctuates from 26/1 to 250/1, and in salmonella from 833,000/1 to 20,000,000/1. In studying the frequency of the formation of L-forms in various species of bacteria it was established that the capability for L-transformation was more expressed in gram negative species (Pr. vulgaris, S. typhosa, S. typhimurium, N. gonorrhea) than in gram positive species (Staph. aureus, Strept. Haemolyticus). For example, following the action of penicillin on S. typhosa, out of 43 strains the formation of typical L-forms was detected in 41, and out of 9 strains of S. typhimurium it was found in 8. Out of 94 strains of gonococcus which were subjected to the L-transforming action of penicillin 40 formed cultures of L-forms and 35 were made up of L-colonies and changed cocci. Other results were obtained during the study of the transforming effect of penicillin on gram positive species of bacteria. Thus out of 64 cultures of hemolytic streptococci the formation of L-forms was observed in 12, including in 8 out of 39 strains of beta-hemolytic streptococci investigated and in 3 out of 26 strains of alpa-hemolytic streptococci. The formation of L-forms in diphtheria bacteria was noted in 13 strains out of 30 which had been passaged many times on a medium with a transforming agent.

The capability for the transformation of bacteria into L-forms depends also on the strain and individual status of each specific population. We followed the individual capability of separate strains for L-transformation and subsequent stabilization in 12 typhoid strains. Each of these strains was inoculated in 40 test tubes of semi liquid Hottinger agar (0.3%) containing 500 active units/ml of penicillin and 10% normal horse serum. The volume of the medium and the amount of inoculated culture were exactly identical (10 ml of medium, 4×10^8 of an 18 hour culture cultivated in Hottinger broth).

It is apparent from the table that the formation of L-forms and their subsequent stabilization fluctuated depending on the strain. Together with

strains in which the capability for L-transformation was sharply expressed (No 5569, 12134, A, 6936) cultures were encountered in which this capability was less expressed (Ty_2, 0-901, H-901).

An analysis of the behavior of the population of all 12 strains testified that each of them was made up of cells which possessed a different degree of sensitivity to the L-transforming action of penicillin and also a different capability for subsequent stabilization in the L-form.

The presence in each specific population of test tubes with the growth of L-colonies along with the absence of bacterial growth was apparently caused by the fact that in the specific portion of the inoculate cells predominated which were distinguished by an increased sensitivity to the L-transforming action of penicillin. In this specific case penicillin was not only the factor inducing the formation of L-forms in cells which were sensitive to its transforming action but was also a sectionalized factor causing the survival of L-forms and the death of bacterial forms. The simultaneous presence in the population of cells, dependent on the growth of bacterial forms, recorded in test tubes with bacterial growth along with the complete absence of L-forms is connected with the fact that in the sown portion of the inoculate a large portion of the cells didn't possess a sensitivity to the L-transforming action of penicillin. Intensive growth of bacterial forms was observed in connection with this. This didn't permit the exposure of the possible occurrence of single L-forms in a specific test tube.

The capability for subsequent stabilization isn't always accompanied by an increased capability for L-transformation. For example, out of 37 test tubes with the growth of L-colonies in strain No 5569 stabilization was observed in 12. In the remaining 25 the intensive growth of bacterial forms was detected together with individual L-colonies. At the same time out of 34 test tubes of the BT_{34} strain with the growth of L-colonies stabilization was noted only in 2, and out of 36 test tubes with the growth of L-colonies of strain No 6936--only in 1.

Thus, stabilization in the form of L-colonies -- the hereditary fixing over again of acquired features of L-forms -- is inherent to a smaller number of cells in the population than the capability for temporary modified changes.

The formation of L-forms depends not only on the individual sensitivity of the cell population to the transforming agent but also on the intensity of the latter's action. In a comparative study of the individual reaction of various strains of S. typhimurium and Staphylococcus aureus to the transforming action of a various intensity of penicillin (fig 1), it was established that during the seeding of strains of S. typhimurium on semiliquid (0,3%) serum agar containing from 8 up to 10,000 active units/ml of penicillin, signi-

ficant fluctuations were observed in the nature of the reaction, depending on the dosage of the antibiotic in the cultural medium. Thus in the initial seeding the transformation of bacteria into L-forms in the 9 cultures studied can be observed in 3 strains (No 79, 73 and 7091) with comparatively high concentrations of the antibiotic -- 125-2000 active units/ml of penicillin. But the formation of these L-colonies was noted in relatively late periods of cultivation -- on the 20th-30th day following seeding. The influence of lower concentrations of the antibiotic (8-250 active units/ml) already in the initial seeding caused the transformation of all 9 strains into forms of heteromorphic growth.

Strains No 5710, 1406, 710 and 2503 on media with higher concentrations of penicillin (250-500 and 10,000 active units/ml) formed nonviable colonies of L-forms which were not subinoculated in subcultures.

The successive passaging of forms of heteromorphic growth on media containing higher concentrations of penicillin was accompanied in the 2nd and 3rd passage by the formation of viable colonies of L-forms, with a good capability for growth in subcultures, in 8 out of the 9 strains tested including the 4 strains described above. In the initial seeding of these four with the specified concentrations of penicillin, the growth of nonviable L-colonies was observed. The passaging of L-forms was accompanied by decreased periods for their incubation. The intensive growth of L-colonies in the 2nd and 3rd passage was noticed on the 6th or 7th day.

Similar results were obtained when studying the individual reaction of various strains of Staphylococcus aureus. All 10 strains used in the experiment can be divided into four groups: 1) strains in which in the first passage clearly expressed zones of formation of L-form colonies were noted which, starting from the zone of growth of unchanged forms, in two cases reached a penicillin concentration of 10,000 active units/ml; 2) strains, also forming colonies of L-forms during initial contact of the culture with penicillin, but having expressed zones of heteromorphic growth (from 1 up to 10-50 active units/ml; 3) strains, forming L-forms only after passaging of the culture in the form of heteromorphic forms; 4) strains, not capable of forming viable L-colonies, but having expressed zones of heteromorphic growth. Identical results were obtained when studying the L-transformation of 8 strains of gonococci (fig 2). Thus strain No 14 under low concentrations of transforming agents grew in the form of rapidly reversing heteromorphic forms. Under high concentrations granular disintegration and death were observed. Strain No 4 didn't form L-forms in the presence of the transforming agent in all the concentrations investigated by us; five other strains (No 6, 13, 12, 10, 7) under low concentrations grew in the form of easily reversing forms of heteromorphic growth, under higher concentrations in the form of characteristic L-colonies,

and finally under the highest concentrations disintegration and death were observed.

In this manner the results of studying L-transformation depending on the intensity of the action testify that within the limits of each species there are strains which do not form L-colonies but are capable of reacting with a temporary transition to heteromorphic growth, strains forming L-colonies after a stay in the phase of heteromorphic growth, and finally, strains which form stable L-forms immediately after the initial action.

CONCLUSIONS

1. The study of the L-transformation in S. typhosa, S. typhimurium, C. diphtheriae, Strept. haemolyticus, Staph. aureus, and N. gonorrhoeae cleared up the existence of a number of general regularities.

2. In all the pathogenic species studied the capability for L-transformation was exposed, however in the gram negative species of S. typhosa, S. typhimurium, and N. gonorrhoeae it was more expressed than in the gram positive species of Strept. haemolyticus, Staph. aureus and C. diphtheriae.

3. The formation of L-forms in pathogenic species depended on the conditions of cultivation, the nature of the action and its intensity. Under equal conditions within the limits of each of the given species there were strains possessing a various capability for L-transformation and subsequent stabilization in the L-form.

4. The study of the capability for L-transformation exposed the heterogeneity of the populations in respect to this property. Along with cells which transformed into L-forms relatively easily under the influence of a transforming agent, cells were encountered with a limited capability for L-transformation which were nonviable, rapidly perishing in the specified conditions.

5. Stable L-forms were formed as a result of the initial reaction to the transforming influence or as a result of the prolonged passaging of diverse forms of heteromorphic growth and nonstable L-forms under conditions of the action of a transforming agent.

6. Nonstable L-forms and forms of heteromorphic growth can be looked at as modification. The mechanisms of formation of stable L-forms have been

studied little, nevertheless in a number of cases their emergence can be looked at as a mutation-selective process in which the transforming agents induce the formation of L-forms and cause the selection of the L-mutants which appear.

LITERATURE

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2. Terranova, T., de Gregorio, P., Arch. Mikrobiol., 1957, Bd. 28, page 126.

[The following English summary appears with the Russian article.]

Many pathogenic species of bacteria are capable of L-transformation. This capacity was more pronounced in the Gram-negative bacteria and less--in the Gram-positive ones. L-form production depended on the culturing conditions, the character of actions used and their intensity. With equal conditions there were strains within the range of the given species, possessing different capacity to L-transformation with a subsequent stabilization in L-form. L-form populations are heterogenic by their capacity to L-transformation. Along with the cells, which are relatively easily transformed into L-form under the effect of transforming agents, there occur cells with a limited capacity to L-transformation and nonviable ones, rapidly perishing in the given conditions.

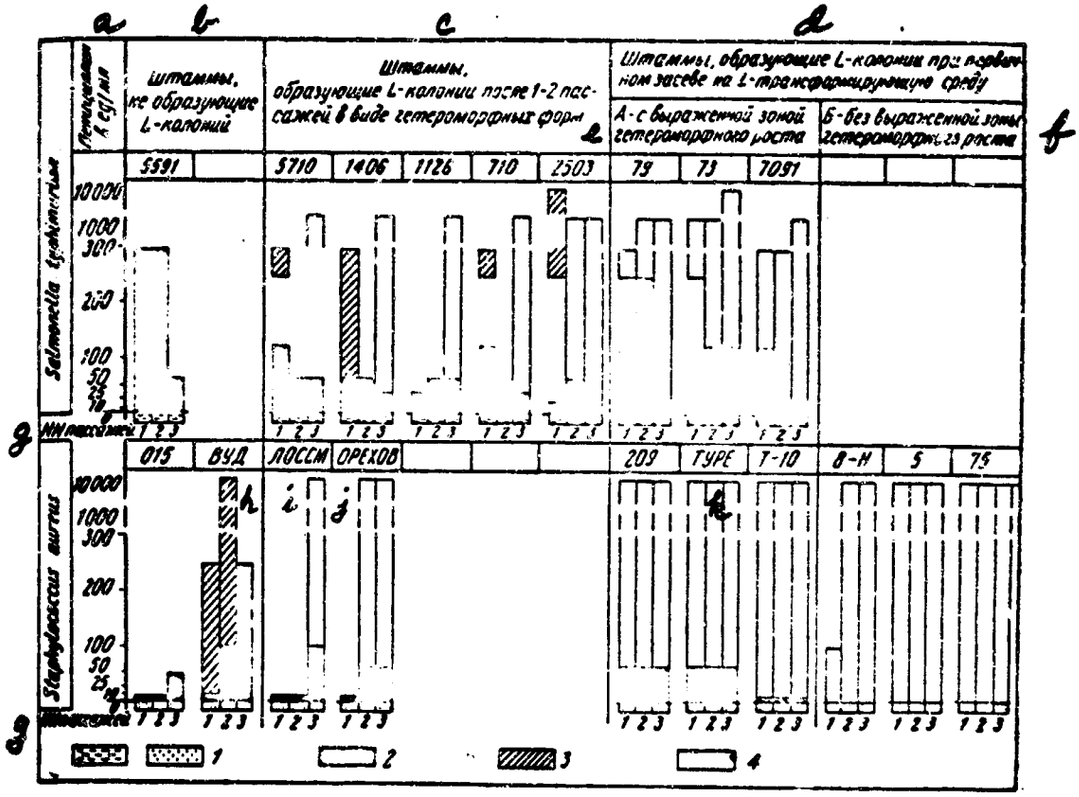
Stable L-forms are produced as a result of the primary reaction to the transforming action, or as a result of prolonged passage of various heteromorphic growth forms and of nonstable L-forms in conditions of the transforming agent action.

Nonstable L-forms may be regarded as modifications. The mechanisms of the stable L-form production are still obscure; nevertheless there is basis for supposition concerning the mutation-selective process of L-transformation, in which the transforming agents induce the appearance of L-forms and simultaneously lead to the selection of the appearing L-mutants.

Capability for L-transformation and subsequent stabilization in the L-form in various typhoid strains.

No. of culture	Number of test tubes			Following storage under laboratory conditions for five months	
	Number inoculated	in which growth of L-colonies was absent	in which there was no growth of bacteria but of L-colonies	L-forms preserved in the form of stable L-colonies	Mixed cultures of L-forms & bacteria, reversion of bacterial forms
5569	40	3	37	12	25
12134	40	4	36	11	25
12304	40	13	27	7	20
9641	40	15	25	5	20
A	40	3	37	12	25
8045	40	6	34	4	30
Ty_2	40	18	22	2	20
O-901	40	16	24	8	16
H-901	40	21	19	1	18
11999	40	13	27	2	25
6936	40	4	36	1	35
BT_{34}	40	6	34	2	32

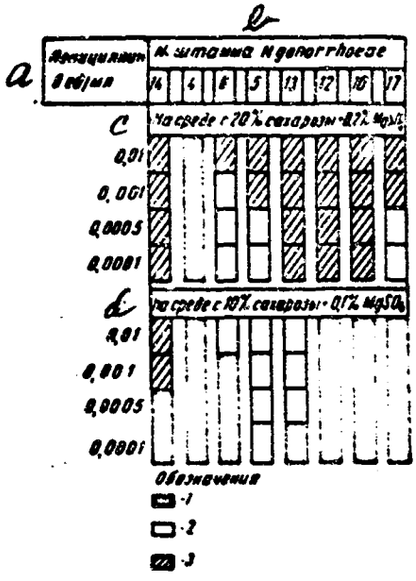
Fig. 1. Comparative characteristics of the individual reaction of various strains of S. typhimurium and Staph. aureus in response to the L-transforming action of penicillin. Legend: 1 - unchanged bacteria and cocci, 2 - heteromorphic forms, 3 - nonviable colonies of L-forms, 4 - viable L-colonies.



a - Penicillin in active units/ml
b - Strains not forming L-colonies
c - Strains forming L-colonies after 1-2 passages in the form of heteromorphic forms
d - Strains forming L-colonies during the initial seeding on a L-transforming medium
e - A - with an expressed zone of heteromorphic growth
f - B - without an expressed zone of heteromorphic growth
g - Number of passages
h - VUD
i - LOSSM
j - OREKHOV
k - TURE

Fig. 2. Characteristics of the individual reaction of various strains of N. gonorrhoeae in response to the L-transforming action of penicillin.

1 - M-forms and cocci
2 - L-forms
3 - granular disintegration and death



a - Penicillin in active units/ml
b - Number of strain of N gonorrhoeae
c - On a medium with 20% saccharose + 0.2% $MgSO_4$
d - On a medium with 10% saccharose + 0.1% $MgSO_4$